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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/33, C07K 14/03, C12N 5/00, C07K 16/08, G01N 33/50, C12Q 1/68, A61K 39/245

A1

(11) International Publication Number: WO 98/03657

(43) International Publication Date: 29 January 1998 (29.01.98)

(21) International Application Number: PCT/EP96/03199

(22) International Filing Date: 19 July 1996 (19.07.96)

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(81) Designated States: US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.

(54) Title: VIRAL INTERLEUKIN-6

(57) Abstract

The present invention relates to viral interleukin-6 (v-IL-6), which can be obtained by recombinant expression of the DNA of human herpesvirus type 8 (HHV-8), and which may be used in diagnosis and treatment of human diseases such as kaposi sarcoma, Castleman's disease, multiple myeloma, kidney cell carcinoma, mesangial proliferative glomerulonephritis or B cell lymphoma.

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Viral Interleukin-6

The present invention relates to viral interleukin-6 (v-IL-6), which can be obtained by recombinant expression of the DNA of human herpesvirus type 8 (HHV-8), and which may be used in diagnosis and treatment of human diseases such as kaposi sarcoma, Castleman's disease, multiple myeloma, kidney cell carcinoma, mesangial proliferative glomerulonephritis or B cell lymphoma.

Kaposi's sarcoma (KS), a multifocal proliferative lesion of uncertain pathogenesis, is highly prevalent among homosexual AIDS patients. Studies with biopsy materials and cultured cells have indicated an important role of growth factors and cellular cytokines, such as basic fibroblast growth factor, interleukin-1β, platelet derived growth factor, interleukin-6 (IL-6), and oncostatin M for the proliferation of spindle cells in KS [1,2]. Several groups found indication for the expression of interleukin-6 (IL-6) receptors in AIDS-KS cells [3] and derived spindle cell lines [4]. As epidemiological evidence had suggested that an infectious agent other than HIV may also be involved in KS pathogenesis, it stirred considerable interest when Chang and colleagues [5] found DNA sequences of a novel herpesvirus in AIDS-KS tissues. Meanwhile, DNA of this virus was consistently found in all epidemiological forms of KS. The new virus, termed human herpesvirus 8 (HHV-8), shows marked sequence homology to *herpesvirus (h.) saimiri*, the prototype of γ_2-herpesviruses; thus HHV-8 appears to be the first human

member of γ_2-herpesviruses (genus rhadinovirus). Cloning HHV-8 DNA from KS tissues and sequencing indicates a genome organization that is generally collinear to *h. saimiri* [6].

In the course of these studies we surprisingly found, adjacent to a dihydrofolate reductase gene, an open reading frame (ORF) with the coding capacity for a 204 amino acid polypeptide with marked homology to mammalian IL-6 (P-value for homology searches with NCBI-BLAST: $P \leq 10\text{-}18$; percent identity/similarity to human IL-6: 24.74%/ 46.91%; to murine: 24.23%/ 47.94%; to porcine: 25.97%/ 52.91%; to bovine: 24.60%/ 49.73%; all alignments were calculated with the GCG software "GAP").

The viral gene product (v-IL-6) has conserved all 4 cysteine residues that are known to be involved in IL-6 disulfide bridging, and it shows a characteristic signal peptide of 19 to 22 amino acids (fig. 1). The area involved in binding of human IL-6 to its receptor has been mapped to the middle of the protein by two groups[7, 8, 9]. Ehlers et al. showed that amino acids 105 to 123 of the human IL-6, as shown in fig. 1 (GFNEEtCLVKIitGLLEFE), are involved in receptor binding. Most remarkably, this region is highly conserved in v-IL-6 (GFNEtsCLkKLadGFFEFE). Identity and similarity of v-IL-6 to the receptor binding region of human IL-6 are 58% and 74%, respectively (fig. 1). This is almost identical with the degree of conservation that can be observed in this receptor binding area of human IL-6 to murine IL-6. As both human IL-6 and murine IL-6 are able to bind to the receptor of the other species (murine IL-6 and human IL-6, respectively), it is likely that v-IL-6 is also able to bind to the human and the murine IL-6 receptor.

Rhadinoviruses frequently acquire genes from their host cell [10]. This HHV-8 ORF however, is the first known example of a viral IL-6 structural homologue. Up to now all cell-homologous genes of rhadinoviruses that have been tested were functional; non-functional genes would most likely have been lost in viral evolution. Thus, the conservation of essential IL-6 features makes it highly suggestive that v-IL-6 is

functional in normal HHV-8 replication or persistence. Since models of paracrine growth stimulation of spindle cells by cytokines, including IL-6 and the related oncostatin M, have been proposed for KS pathogenesis, the finding of the v-IL-6 gene in HHV-8 lends support to the hypothesis that HHV-8 is causally related to this multifocal proliferation.

The present invention therefore relates to:

a) Viral interleukin-6 (v-IL-6), which can be obtained by recombinant expression of the DNA of HHV-8.

b) A polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2.

c) A fragment of v-IL-6, having the capability of binding to an IL-6 receptor and comprising the amino acid sequence GFNEtsCLkKLadGFFEFE.

d) A fragment as defined in b1, which essentially comprises the amino acid sequence GFNEtsCLkKLadGFFEFE.

e) A fragment as defined in c or d, which binds to a human IL-6 receptor.

f) A polypeptide having the amino acid sequence displayed in fig. 2.

g) Mutants and variants of v-IL-6 or of the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, which mutants and variants are obtained by conventional amino acid substitutions or deletions, with the proviso that these mutants and variants are functionally equivalent to v-IL-6.

h) Fragments of v-IL-6, or of the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, characterized in that they are able to competively inhibit the biological activity of IL-6 in a suitable assay system.

i) An isolated nucleic acid coding for v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2. A preferred embodiment is the nucleic acid having the nucleotide sequence of fig.2. Furthermore, an isolated nucleic acid, hybridizing to the abovementioned nucleic acids under stringent conditions and encoding functionally active v-IL-6 shall be comprised.

k) Monoclonal or polyclonal antibodies directed against v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2.

l) Testkit for the detection of v-IL-6 in a sample, comprising one or more of the above monoclonal or polyclonal antibodies.

m) Testkit for the detection of antibodies against v-IL-6 comprising v-IL-6 and/or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, and/or mutants and variants of v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2 and/or fragments of v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2.

n) Testkit for the detection of v-IL-6 DNA or RNA, comprising a nucleic acid which codes for v-IL-6, or which hybridizes to the aforementioned nucleic acid and encodes functionally active v-IL-6.

o) A medicament comprising as an active ingredient a monoclonal antibody or polyclonal antibodies directed against v-IL-6, or a polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, or mutants, variants or fragments of v-IL-6 or the aforementioned polypeptide. In another embodiment, the medicament may comprise as an active ingredient a nucleic acid encoding v-IL-6.

p) A cell culture growth medium, comprising as an active ingredient v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2 , or mutants, variants or fragments of v-IL-6 or the aforementioned polypeptide.

q) A process of manufacturing v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, or mutants and variants, or fragments of v-IL-6 or the aforementioned polypeptide.

r) A process of manufacturing a medicament, wherein the active ingredient is combined with suitable excipients and/or other auxilliary compounds according to common knowledge of those skilled in the art. .

s) A process of manufacturing a medicament comprising as an active ingredient monoclonal or polyclonal antibodies directed against v-IL-6, or a polypeptide comprising v-IL-6, or mutants, variants or fragments of v-IL-6, or a nucleic acid encoding v-IL-6 for the treatment of kaposi sarcoma, Castleman's disease, multiple myeloma, kidney cell carcinoma, mesangial proliferative glomerulonephritis or B cell lymphoma.

t) A process of diagnosing an HHV-8 infection comprising the in vitro detection of v-IL-6 antigen, v-IL-6 DNA, v-IL-6 RNA or antibodies against v-IL-6.

u) A process of diagnosing the HHV-8 associated disorders kaposi sarcoma, Castleman's disease or body cavity based lymphomas (BCBL) through the diagnosis of an HHV-8 infection as described above.

v) A process of growing cells in culture, characterized in that v-IL-6 or the polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2, or mutants and variants, or fragments of v-IL-6 or the aforementioned polypeptide, or mixtures of these compounds are contained in the growth medium. In a preferred process these cells are B-lymphocytes, hybridomas, hemopoetic cells or endothelial cells.

The sequence shown in fig.2 was generated by first subcloning shotgun fragments of lambda clone G16 into commercially available plasmid pBS KS- (Stratagene, San Diego, califormia). Resulting plasmids were purified using a commercially available kit (Qiagen, Hilden, Germany) and sequenced on an automated sequencing system (A377, Applied Biosystems GmbH, Weiterstadt, Germany) using the recommendations of the manufacturer. The sequence was determined on both strands, using standard primers for shotgun clones, and gene specific primers for further analysis. In addition to showing the coding sequence of the interleukin-6 homologue of human herpesvirus 8, the deduced amino acid sequence, in one and three letter code, is shown in the sequence listing below.

The present invention is further described in the claims.

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Legends:

Figure 1:

Alignment of the sequences of the predicted protein precursor of the HHV-8 IL-6 gene with human and mouse IL-6. Amino acids identical in all three proteins are indicated by an asterisk, cysteine residues involved in disulfide bridging are marked with an arrowhead. Upper case letters symbolize amino acids conserved according to the criteria defined by M. Dayhoff.

Figure 2:

Nucleic acid sequence encoding v-IL-6 and corresponding amino acid sequence.

Claims:

1. Viral interleukin-6 (v-IL-6), which can be obtained by recombinant expression of the DNA of HHV-8.

2. A polypeptide, which can be obtained by recombinant expression of the DNA of HHV-8, and which comprises the amino acid sequence displayed in fig. 2.

3. A polypeptide having the amino acid sequence displayed in fig. 2.

4. A fragment of v-IL-6, having the capability of binding to an IL-6 receptor and comprising the amino acid sequence GFNEtsCLkKLadGFFEFE.

5. A fragment as claimed in claim 4, which essentially comprises the amino acid sequence GFNEtsCLkKLadGFFEFE.

6. A fragment as claimed in claim 4 or 5, which binds to a human IL-6 receptor.

7. Mutants and variants of v-IL-6 as claimed in claim 1, or of the polypeptide as claimed in claim 2, which mutants and variants are obtained by conventional amino acid substitutions or deletions, with the proviso that these mutants and variants are functionally equivalent to v-IL-6.

8. Fragments of the v-IL-6 as claimed in claim 1, or the polypeptide as claimed in claim 2 or 3, characterized in that they able to competively inhibit the biological activity of IL-6 in a suitable assay system.

9. An isolated nucleic acid coding for v-IL-6 as claimed in claim 1.

10. An isolated nucleic acid coding for the polypeptide as claimed in claim 2.

11. An isolated nucleic acid having the nucleotide sequence displayed in fig. 2.

12. An isolated nucleic acid, hybridizing under stringent conditions to the nucleic acid as claimed in one or more of the claims 9 to 11, encoding functional v-IL-6.

13. Monoclonal or polyclonal antibodies directed against v-IL-6 as claimed in claim 1, or the polypeptide as claimed in claim 2 and/or 3.

14. Testkit for the detection of v-IL-6 in a sample, comprising an antibody as claimed in claim 16.

15. Testkit for the detection of antibodies against v-IL-6, comprising v-IL-6 as claimed in claim 1 and/or the polypeptide as claimed in claim 2 or 3 or both, claims 2 and 3, and/or mutants and variants of v-IL-6 as claimed in claim 7, and/or fragments of v-IL-6 as claimed in claim 4-6 or 8.

16. Testkit for the detection of v-IL-6 DNA or RNA, comprising a nucleic acic as claimed in one or more of the claims 9 to 12.

17. A medicament comprising as an active ingredient the antibody as claimed in claim 13.

18. A medicament comprising as an active ingredient v-IL-6 as claimed in claim 1 and/or the polypeptide as claimed in claim 2 or 3, and/or mutants and variants of v-IL-6 as claimed in claim 7, and/or fragments of v-IL-6 as claimed in claim 4-6 or 8.

19. A medicament comprising as an active ingredient the nucleic acid as claimed in in one or more of claims 9 to 12.

20. A cell culture growth medium, comprising as an additional active ingredient v-IL-6 as claimed in claim 1, or the polypeptide as claimed in claim 2 or 3, or mutants and variants as claimed in claim 7, or fragments as claimed in claim 8, or mixtures of these substances.

21. A process of manufacturing v-IL-6 as claimed in claim 1, or the polypeptide as claimed in claim 2 or 3, or mutants and variants as claimed in claim 7, or fragments as claimed in claim 4-6 or 8.

22. A process of manufacturing a medicament, wherein v-IL-6 as claimed in claim 1, or the polypeptide as claimed in claim 2 or 3, or mutants and variants as claimed in claim 7, or fragments as claimed in claim 8 are combined with suitable excipients and/or other auxilliary compounds.

23. A process of manufacturing a medicament comprising as an active ingredient monoclonal or polyclonal antibodies directed against v-IL-6, or a polypeptide comprising v-IL-6, or mutants, variants or fragments of v-IL-6, or a nucleic acid encoding v-IL-6 for the treatment of kaposi sarcoma, Castleman's disease, multiple myeloma, kidney cell carcinoma, mesangial proliferative glomerulonephritis or B cell lymphoma.

24 An process of diagnosing an HHV-8 infection comprising the in vitro detection of v-IL-6 antigen, v-IL-6 DNA, v-IL-6 RNA or antibodies against v-IL-6.

25. A process of diagnosing the HHV-8 associated disorders kaposi sarcoma, Castleman's disease or body cavity based lymphomas (BCBL) through the diagnosis of an HHV-8 infection as claimed in claim 24.

26. A process of growing cells in culture, characterized in that v-IL-6 as claimed in claim 1, or the polypeptide as claimed in claim 2 or 3, or mutants and variants as claimed in claim 7, or fragments as claimed in claim 4-6 or 8, or mixtures of these compounds are contained in the growth medium.

27. The process as claimed in claim 26, wherein the cells are B-lymphocytes, hybridomas, hemopoetic cells or endothelial cells.

Fig. 1:

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                1                                                      56
Il6 human       MnsFStsaFgPVAFsLGLLLVlpaAFPapvppgeDskDvaaPhRQpLTsSErIDkq
Il6 mouse       MkFLSaRdFhPVAF.LGLMLVttTAFPtsqvrRGDFtEdttPnRpVyTtSQ.VGgl
Il6 hhv8        McWFklWsL....LlVGsLLVsgT........RGkLpDapefeKDLLi......qr
 Consensus      *                 *  **

               57                                                     112
Il6 human       IrYILdgIsaLRKEtCNKsnMCeSskEALAENNLnLPkMaEkDGCFQsGFNEEtCL
Il6 mouse       IthVLWeIvEMRKELCNgnSdCmnndDALAENNLKLPeIqrnDGCYQtGYNQEiCL
Il6 hhv8        LnWMLWvIdEcfRDLCyRtGICkGilEpaAifhLKLPaInDtDhCgliGFNEtsCL
 Consensus          * *       *     *       *   * **     * *   * *   **
                               ^     ^                      ^         ^

               113                                                    168
Il6 human       VKIitGLLEFEVYLEYLqNrF.EsSeEqARaVQMsTKvLIQFLQkkaKNLdaIttP
Il6 mouse       LKIssGLLEYhsYLEYMkNnLkDnkkDkARVLQrdTeTLIHIFnQEVKDLhKIvlP
Il6 hhv8        kKLadGFFEFEVlFkFLtteF.GkSvinvdVMELlTKTLgwdIQEELnkLtKthys
 Consensus       *   *  *                              *  *          *

               169                                                    223
Il6 human       dPttNASLLtKLQAQnQWLqdmTtHLILRSFkEFLqssLRaLRQM.........
Il6 mouse       tPisNAlLtDKLESQKEWLRtkTiQFILKSLEEFLkvtLRstRQt.........
Il6 hhv8        pPkfDrGLLGRLQGlKyWVRhfasfYVLsaMEkFagqaVRvLdsIpdvtpdvhdk
 Consensus       *     *   *     *         *     *     *
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Fig. 2:

SEQUENCE LISTING

1.	Sequence characteristics:	
	1.1. Length:	612 base pairs
	1.2. Type:	Nucleic Acid
	1.3. Strandedness:	Double stranded
	1.4. Topology:	Linear
2.	Molecule type:	Genomic DNA
3.	Description:	Human herpesvirus 8 interleukin-6 gene
4.	Hypothetical:	No
5.	Anti-sense:	No
6.	Original source:	Kaposi Sarkoma from HIV positive donor
7.	Organism:	Human herpesvirus 8

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  1  ATG TGC TGG TTC AAG TTG TGG TCT CTC TTG CTG GTC GGT TCA CTG
  1   M   C   W   F   K   L   W   S   L   L   L   V   G   S   L
  1  Met Cys Trp Phe Lys Leu Trp Ser Leu Leu Leu Val Gly Ser Leu

 46  CTG GTA TCT GGA ACG CGG GGC AAG TTG CCG GAC GCC CCC GAG TTT
 16   L   V   S   G   T   R   G   K   L   P   D   A   P   E   F
 16  Leu Val Ser Gly Thr Arg Gly Lys Leu Pro Asp Ala Pro Glu Phe

 91  GAA AAG GAT CTT CTC ATT CAG AGA CTC AAT TGG ATG CTA TGG GTG
 31   E   K   D   L   L   I   Q   R   L   N   W   M   L   W   V
 31  Glu Lys Asp Leu Leu Ile Gln Arg Leu Asn Trp Met Leu Trp Val

136  ATC GAT GAA TGC TTC CGC GAC CTC TGT TAC CGT ACC GGC ATC TGC
 46   I   D   E   C   F   R   D   L   C   Y   R   T   G   I   C
 46  Ile Asp Glu Cys Phe Arg Asp Leu Cys Tyr Arg Thr Gly Ile Cys

181  AAG GGT ATT CTA GAG CCC GCT GCT ATT TTT CAT CTG AAA CTA CCA
 61   K   G   I   L   E   P   A   A   I   F   H   L   K   L   P
 61  Lys Gly Ile Leu Glu Pro Ala Ala Ile Phe His Leu Lys Leu Pro

226  GCC ATC AAC GAT ACT GAT CAC TGC GGG TTA ATA GGA TTT AAT GAG
 76   A   I   N   D   T   D   H   C   G   L   I   G   F   N   E
 76  Ala Ile Asn Asp Thr Asp His Cys Gly Leu Ile Gly Phe Asn Glu

271  ACT AGC TGC CTT AAA AAG CTC GCC GAT GGC TTT TTT GAA TTC GAG
 91   T   S   C   L   K   K   L   A   D   G   F   F   E   F   E
 91  Thr Ser Cys Leu Lys Lys Leu Ala Asp Gly Phe Phe Glu Phe Glu

316  GTG TTG TTT AAG TTT TTA ACG ACG GAG TTT GGA AAA TCA GTG ATA
106   V   L   F   K   F   L   T   T   E   F   G   K   S   V   I
106  Val Leu Phe Lys Phe Leu Thr Thr Glu Phe Gly Lys Ser Val Ile

361  AAC GTG GAC GTC ATG GAG CTT CTG ACG AAG ACC TTA GGA TGG GAC
121   N   V   D   V   M   E   L   L   T   K   T   L   G   W   D
121  Asn Val Asp Val Met Glu Leu Leu Thr Lys Thr Leu Gly Trp Asp

406  ATA CAG GAA GAG CTC AAT AAG CTG ACT AAG ACG CAC TAC AGT CCA
136   I   Q   E   E   L   N   K   L   T   K   T   H   Y   S   P
136  Ile Gln Glu Glu Leu Asn Lys Leu Thr Lys Thr His Tyr Ser Pro

451  CCC AAA TTT GAC CGC GGT CTA TTA GGG AGG CTT CAG GGA CTT AAG
151   P   K   F   D   R   G   L   L   G   R   L   Q   G   L   K
151  Pro Lys Phe Asp Arg Gly Leu Leu Gly Arg Leu Gln Gly Leu Lys

496  TAT TGG GTG AGA CAC TTT GCT TCG TTT TAT GTT CTG AGT GCA ATG
166   Y   W   V   R   H   F   A   S   F   Y   V   L   S   A   M
166  Tyr Trp Val Arg His Phe Ala Ser Phe Tyr Val Leu Ser Ala Met
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541  GAA AAG TTT GCA GGT CAA GCG GTG CGT GTT TTG GAC TCT ATC CCA
181   E   K   F   A   G   Q   A   V   R   V   L   D   S   I   P
181  Glu Lys Phe Ala Gly Gln Ala Val Arg Val Leu Asp Ser Ile Pro

586  GAC GTG ACT CCT GAC GTC CAC GAT AAG
196   D   V   T   P   D   V   H   D   K
196  Asp Val Thr Pro Asp Val His Asp Lys
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INTERNATIONAL SEARCH REPORT

[redacted] onal Application No
[redacted]/EP 96/03199

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/33 C07K14/03 C12N5/00 C07K16/08 G01N33/50
C12Q1/68 A61K39/245

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N G01N C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	J VIROL, JAN 1997, 71 (1) P839-42, UNITED STATES, XP000645323 NEIPEL F ET AL: "Human herpesvirus 8 encodes a homolog of interleukin-6." see the whole document ---	1-3,6-27
P,X	PROC NATL ACAD SCI U S A, DEC 10 1996, 93 (25) P14862-7, UNITED STATES, XP000645332 RUSSO JJ ET AL: "Nucleotide sequence of the Kaposi sarcoma-associated herpesvirus (HHV8)." see figure 1; table 1 --- -/--	1-3,6-12

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	SCIENCE, DEC 6 1996, 274 (5293) P1739-44, UNITED STATES, XP002027822 MOORE PS ET AL: "Molecular mimicry of human cytokine and cytokine response pathway genes by KSHV." see figure 1B ---	1-3,6-12
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, vol. 93, June 1996, WASHINGTON US, pages 6641-6646, XP002027823 ZHONG W ET AL.: "Restricted expression of Kaposi sarcoma associated herpesvirus (human herpesvirus 8) genes in Kaposi sarcoma" -----	

INTERNAT[redacted] SEARCH REPORT

International application No.
[redacted]./EP 96/03199

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 4,5
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Claims not searched. The amino acid sequence given in these claims lacks clarity, since the single letter code used does not comply with the official code generally used.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.